Original Research Article

SCREENING AND IN SILICO VALIDATION OF ANTI-MICROBIAL PEPTIDES DERIVED FROM LYSOSTAPHIN, ENTERO AND ENDOLYSIN

Running title: *In silico* screening of antimicrobial peptides

ABSTRACT

Introduction: Antimicrobial peptides (AMPs) are small molecules which are known to exert destructive effects upon pathogenic microorganisms. AMPs can be designed from proteins obtained from various sources employing *in silico* tools. The predicted peptides can be further validated under *in vitro* conditions to deduce their antimicrobial activity. The present study is an attempt to identify the antimicrobial peptides from proteins lysostaphin, entero and endolysin.

Materials and Methods: Peptidoglycan hydrolases s lysostaphin (AAB53783.1), enterolysin (AGG79281.1), and endolysin (YP_009901016.1) were selected for the study based on an extensive text mining process. Protein sequences were retrieved from the NCBI (National Centre for Biotechnology Information) database in the FASTA format (https://www.ncbi.nlm.nih.gov/protein/).

Results and Discussion: Three antimicrobial peptides were identified in the antimicrobial protein lysostaphin, of which two were found to exhibit antibiofim

property and one in enterolysin, endolysin. Among the four AMPs exhibiting anti-biofilm property, three were found to exhibit antifungal property. All the above mentioned AMPs were non-cell penetrating and non-toxic.

Conclusion: The present study predicts AMPs from lysostaphin, entero and endolysins. These peptides were predicted to exhibit the antifungal and anti-biofilm properties. Further validation using *in vitro* and *in vivo* experimental designs could enable us to choose the best AMP among the predicted pool of peptides.

Keywords: Antimicrobial peptides, anti-fungal, biofilm, lysostaphin, entero, endolysins, novel peptides

INTRODUCTION

Antimicrobial drug resistance has emerged as a global threat in recent years. Novel strategies have been developed to identify bioactive leads which can be used as a therapeutic modality against microbial pathogens, with a special emphasis on the drug resistant groups. Numerous reports have suggested the emergence of novel drug resistant pathogens in dental settings (1); (2). Phytocompounds, compounds from marine and animal sources and non-antibiotic drugs were repurposed for use as antimicrobial agents (3); (4); (5); (6).

Antimicrobial peptides are small molecules which have opened a new era of peptide therapeutics. These are oligopeptides with different numbers of amino acid residues. They have been shown to have a broad spectrum of activity which ranges from anti-bacterial, anti-viral, anti-parasitic etc (7). The major class of peptides are as follows: cationic peptides, anionic peptides, cationic amphipathic peptides, host defense peptides, alpha helical peptides etc., (7). In line with these facts three antimicrobial proteins were selected by performing text mining process. The proteins lysostaphin (AAB53783.1), enterolysin (AGG79281.1), and endolysin (YP_009901016.1) were selected because their role in targeting bacterial pathogens have been adequately discussed by several

researchers. Despite the presence of evidence for their activity among gastroinstestinal pathogens, their role in combating oro-dental pathogens remains scarce. Our team has extensive knowledge and research experience that has translate into high quality publications[1–5]

Hence, these antimicrobial proteins were selected to perform the preliminary predictions to deduce the anti-biofim and anti-fungal properties.

MATERIALS AND METHODS

Three peptidoglycan hydrolases, namely lysostaphin (AAB53783.1), enterolysin (AGG79281.1), and endolysin (YP_009901016.1) were selected for the study. The protein sequences of the proteins were retrieved from the NCBI (National Centre for Biotechnology Information) database in the FASTA format (https://www.ncbi.nlm.nih.gov/protein/) (data not shown).

Antimicrobial peptide identification

Antimicrobial peptide analysis (AMPA) is a web based application employed for identifying and assessing the antimicrobial domains in a protein. The source is used to design and develop peptide based drugs against microbial pathogens[6]**8,9**[7]**.**

Anti-biofilm property

dPABB [https://ab-openlab.csir.res.in/abp/antibiofilm/feature.php] (design Peptides Against Bacterial Biofilms) algorithm is based on the SVM which is used to identify anti-biofilm peptides based on their amino acid composition, selected residue and position of the residues. The scores generated for each of the peptide molecules are then used to ascertain the anti-biofilm property[8]**.**

Antifungal property:

The tool used in silico prediction of antimicrobial peptides for its antifungal property is Antifp (https://webs.iiitd.edu.in/raghava/antifp). The module allows users to predict single or multiple sequences for its antifungal properties. The tool can be used for designing peptides and scanning protein sequences to identify peptides and their mutant analogs followed by the screening for antifungal property[9]**.**

Cell penetrating property:

Identification of newer peptide molecules with the ability to penetrate cells using high throughput methods is known to consume time as well as labour. The in silico screening procedures coupled with experimental validation is considered to be more feasible and cost-effective. The results could be replicated in in vitro conditions with much ease and confidence. CellPPD (https://webs.iiitd.edu.in/raghava/cellppd/algo.php) is one such standalone application developed to predict and design cell penetrating peptide molecules (12, 13).

Toxicity prediction:

Prediction of toxicity of peptides is a vital step in designing antimicrobial peptides. The ToxinPred (https://webs.iiitd.edu.in/raghava/toxinpred/multi_submit.php) tool has been used in the present study. The algorithm identifies certain amino acid residues such as Cys, His, Asn and Pro and their placements at various positions which makes them toxic. ToxinPred can be used to predict whether the designed peptide is toxic or non-toxic, consequences of mutations on toxicity and identification of toxic regions in a protein[10]**.**

Results and discussion

Lysostaphin is a potent antimicrobial agent, which falls under the major class of proteins called bacteriocins. Bacteriocins are antimicrobial proteins exhibiting bactericidal activity against other bacterial species. This endopeptidase derived from

Staphylococcus simulans **was found to break the peptidoglycan bridge (15). Enterolysin is a protein purified from** ***Enterococcus faecalis.*** **The protein was found to have an inhibitory effect on** ***Enterococci, Lactococci*** **and** ***Lactobacilli*** **(16). Endolysins are cell wall hydrolyzing enzymes synthesized by phages. These enzymes fall into 4 classes: glycosidases, transglycosylases, amidases, endopeptidases. More than thousands of endolysins are identified from uncultured bacteriophages (17). Several studies have been conducted by the authors to reveal the effects of antimicrobial phytocompounds or bioactive compounds against dental pathogens (18).**

The present study identified six AMPs as predicted by the AMPA tool from the antimicrobial proteins mentioned above (Figure 1). Further, these peptide sequences were used predict the anti-biofilm, anti-fungal properties. In addition, the cell penetration ability and toxicity were also predicted. Out of three peptides of lysostaphin 2 were found to exhibit antibiofilm property and one was found to exhibit antifungal property. Among the peptides of enterolysin one peptide was found to exhibit both antifungal and antibacterial property. A similar observation was seen with endolysin where one peptide was found to exhibit anti-biofilm and anti-fungal property. All the peptides except one of the lysostaphin was found to be non-cell penetrating. Almost all the peptides observed were predicted to be non-toxic in nature (Table 1). The physicochemical properties of the peptides identified are given in Table 2. These proteins have been used or tested against common pathogens associated with hospital acquired infections. The present study is first of its kind to identify the potential properties of a antimicrobial peptides that can be used dental settings for treatment of diseases caused by oral pathogens. Nevertheless, more evidences have to be accumulated so as to gain knowledge about the molecular pathways and interactions between the virulence proteins and the peptides predicted. Our team has extensive knowledge and experience in the field of translational medicine employing phytocompounds as therapeutic leads, characterization of drug resistant pathogens and designing small, synthetic molecules to be used as drugs for the treatment of oral and other common diseases (19-36).

Conclusion

The present study predicted antimicrobial peptides in commonly known antimicrobial proteins. The workflow adopted in this study will serve the purpose of predicting the physicochemical properties of the antimicrobial peptides with a special emphasis on revealing unique features such as anti-biofilm and anti-fungal properties which is very crucial while selecting molecules against dental pathogens.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

1. **Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. An insight into the emergence of *Acinetobacter baumannii* as an oro-dental pathogen and its drug resistance gene profile - An in silico approach. Heliyon. 2018;4(12):e01051.**

2. **Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species. Arch Oral Biol. 2018;94:93-98.**

3. **Balamithra, S., Girija, S., & Priyadharsini, J. V. An in silico Analysis of Protein Targeted by Glycyrrhizin in Common Dental Pathogens. Journal of Pharmaceutical Research International, 2020; 32(15), 170-178.**

4. **Cibikkarthik.T, Smiline Girija.A.S, Vijayashree Priyadharsini.J. An in silico approach to detect potential targets of catechin in red complex pathogens. JCR. 2020; 7(12): 3328-3331.**

5. Nandhini, J. S. T., Girija, A. S. S., & Priyadharsini, J. V. (2020). Virtual Screening to Identify the Protein Targets in Common Dental Pathogens Interacting with Menthol. Journal of Pharmaceutical Research International, 32(21), 25-31.

6. Vivek Babu B, A.S.Smiline Girija, J.Vijayashree Priyadharsini. An in silico approach to identify the protein targets of common dental pathogens targeted by genistein. JCR. 2020; 7(12): 3340-3348.

7. Bahar, AA and Ren, D. Antimicrobial peptides. Pharmaceuticals (Basel, Switzerland), 2013; 6(12), 1543–1575.

8. Torrent M, Di Tommaso P, Pulido D, Nogués MV, Notredame C, Boix E, Andreu D. AMPA: an automated web server for prediction of protein antimicrobial regions. Bioinformatics. 2012;28(1):130-1.

9. Torrent M, Nogués VM, Boix E. A theoretical approach to spot active regions in antimicrobial proteins. BMC Bioinformatics. 2009;10:373.

10. Sharma A, Gupta P, Kumar R, Bhardwaj A. dPABBs: A Novel in silico Approach for Predicting and Designing Anti-biofilm Peptides. Sci Rep. 2016;6:21839.

11. Agrawal P, Bhalla S, Chaudhary K, Kumar R, Sharma M, Raghava GPS. In Silico Approach for Prediction of Antifungal Peptides. Front Microbiol. 2018;9:32.

12. Gautam A, Chaudhary K, Kumar R, Raghava GP. Computer-Aided Virtual Screening and Designing of Cell-Penetrating Peptides. Methods Mol Biol. 2015;1324:59-69.

13. Gautam A, Chaudhary K, Kumar R, Sharma A, Kapoor P, Tyagi A; Open source drug discovery consortium, Raghava GP. In silico approaches for designing highly effective cell penetrating peptides. J Transl Med. 2013;11:74.

14. Gupta S, Kapoor P, Chaudhary K, Gautam A, Kumar R; Open Source Drug Discovery Consortium, Raghava GP. In silico approach for predicting toxicity of peptides and proteins. PLoS One. 2013;8(9):e73957.

15. Jayakumar J, Kumar VA, Biswas L, Biswas R. Therapeutic applications of lysostaphin against Staphylococcus aureus. J Appl Microbiol. 2020 Dec 31 [Epub ahead of print].

16. Nilsen T, Nes IF, Holo H. Enterolysin A, a cell wall-degrading bacteriocin from Enterococcus faecalis LMG 2333. Appl Environ Microbiol. 2003;69(5):2975-84.

17. Fernández-Ruiz I, Coutinho FH, Rodriguez-Valera F. Thousands of Novel Endolysins Discovered in Uncultured Phage Genomes. Front Microbiol. 2018;9:1033.

18. Gutiérrez, D., Fernández, L., Rodríguez, A., and García, P. (2018). Are phage lytic proteins the secret weapon to kill *Staphylococcus aureus*? *mBio* 9, e1923-17.

[1] PradeepKumar AR, Shemesh H, Jothilatha S, Vijayabharathi R, Jayalakshmi S, Kishen A. Diagnosis of Vertical Root Fractures in Restored Endodontically Treated Teeth: A Time-dependent Retrospective Cohort Study. J Endod 2016;42:1175–80.

[2] Dhinesh B, Isaac JoshuaRamesh Lalvani J, Parthasarathy M, Annamalai K. An assessment on performance, emission and combustion characteristics of single cylinder diesel engine powered by Cymbopogon flexuosus biofuel. Energy Convers Manage 2016;117:466–74.

[3] Lekha L, Kanmani Raja K, Rajagopal G, Easwaramoorthy D. Schiff base complexes of rare earth metal ions: Synthesis, characterization and catalytic activity for the oxidation of aniline and substituted anilines. J Organomet Chem 2014;753:72–80.

[4] Soh CL, Narayanan V. Quality of life assessment in patients with dentofacial deformity undergoing orthognathic surgery—A systematic review. Int J Oral Maxillofac Surg 2013;42:974–80.

[5] Krishnan V, Lakshmi T. Bioglass: A novel biocompatible innovation. J Adv Pharm Technol Res 2013;4:78–83.

[6] Torrent M, Di Tommaso P, Pulido D, Nogués MV, Notredame C, Boix E, et al. AMPA: an automated web server for prediction of protein antimicrobial regions. Bioinformatics 2012;28:130–1.

[7] Torrent M, Nogués VM, Boix E. A theoretical approach to spot active regions in antimicrobial proteins. BMC Bioinformatics 2009;10:373.

[8] Sharma A, Gupta P, Kumar R, Bhardwaj A. dPABBs: A Novel in silico Approach for Predicting and Designing Anti-biofilm Peptides. Scientific Reports 2016;6. https://doi.org/10.1038/srep21839.

[9] Agrawal P, Bhalla S, Chaudhary K, Kumar R, Sharma M, Raghava GPS. In Silico Approach for Prediction of Antifungal Peptides. Frontiers in Microbiology 2018;9.

https://doi.org/10.3389/fmicb.2018.00323.
[10] Gupta S, Kapoor P, Chaudhary K, Gautam A, Kumar R, Raghava GPS, et al. In Silico Approach for Predicting Toxicity of Peptides and Proteins. PLoS ONE 2013;8:e73957. https://doi.org/10.1371/journal.pone.0073957.

20. Vijayashree Priyadharsini J. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. J Periodontol. 2019;90(12):1441-1448.

Figure 1: The schematic representation of the workflow of protocol used in the present study

Table 1: The list of antimicrobial peptides predicted from Lysostaphin, Enterolysin and Endolysin, their anti-biofilm and anti-fungal properties

Antimicrobial protein	Antimicrobial peptide	Anti-biofilm property	SVM score	Anti-fungal property	Score	Cell penetrating property	Toxicity
Lysostaphin	KKTKNNYYTRPL	Inactive	-0.24	Non-antifungal	-0.117	CPP	Non-toxic
	QWYMHLSKYNVKV	Active	0.28	Antifungal	-0.15	Non-CPP	Non-toxic
	RIYLPVRTWNKSTNT	Active	0.02	Non-antifungal	-0.31	Non-CPP	Non-toxic
Enterolysin	TNVRYGLRVLGG	Inactive	-0.13	Non-antifungal	-0.17	Non-CPP	Non-toxic
	AYYRSQTTKRSGWLKV	Active	0.31	Antifungal	-0.14	Non-CPP	Non-toxic

Endolysin	**WTYYHNPKTGKREKSKGLLNRRKVEYK**	**Active**	**1.28**	**Antifungal**	**-0.39**	**Non-CPP**	**Non-toxic**

Table 2: Physiochemical properties of the antimicrobial peptides

Antimicrobial peptide	Hydrophobicity	Hydropathicity	Hydrophilicity	Molecular weight
KKTKNNYYTRPL	**-0.52**	**-2.08**	**0.43**	**1525.94**
QWYMHLSKYNVKV	**-0.15**	**-0.63**	**-0.61**	**1696.20**
RIYLPVRTWNKSTNT	**-0.31**	**-0.94**	**-0.15**	**1849.34**

TNVRYGLRVLGG	-0.14	0.02	-0.26	1304.70
AYYRSQTTKRSGWLKV	-0.34	-1.09	0.01	1944.45
WTYYHNPKTGKREKSKGLLN RRKVEYK	-0.49	-1.91	0.63	3381.31